

# Specific depletion of the house dust mite allergen Der p 1 by cereal flour prolamins

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**Background:** Quantitation of Der 1 and Der 2 in dust samples by specific monoclonal antibodies is a method used increasingly to evaluate mite allergen exposure. The level of Der 1 has been proposed as a risk factor for sensitization.

**Aim:** We report a drastic decrease in the Der 1/Der 2 ratio when dust samples are collected in bakeries.

**Methods:** Wheat flour and purified mites were extracted simultaneously; levels of Der p 1 and Der p 2 and cysteine protease activity were determined by ELISA and inhibition experiments.

**Results:** High titers of Der 2, but only trace amounts of Der p 1, were detected in dust samples collected from bakeries. Both the level and proteolytic activity of Der p 1 appeared greatly decreased when mites and wheat flour were coextracted.

**Conclusions:** Group 1 protein was found to be masked by flour components, resulting in an underestimation of the mite content in bakery dust. This problem was not found for group 2 allergen. (*J ALLERGY CLIN IMMUNOL* 1996;97:963-5.)

**Key words:** Dust mite allergen, Der p 1, cereal flour prolamins, proteolytic activity, allergenic exposure

Protein groups Der 1 and Der 2 include major *Dermatophagoides* allergens.<sup>1</sup> Their quantitation in dust samples by specific monoclonal antibodies is a method used increasingly to evaluate mite allergen exposure, and the level of Der 1 has been proposed as a risk factor for sensitization. In dust samples Der 1 is usually present at higher levels than Der 2, and a roughly constant ratio (micrograms of Der 1 per microgram of Der 2  $\approx$  1.6) has been determined after screening more than 500 dust samples from different origins.<sup>2</sup> In contrast, we report here a drastic decrease in the Der 1/Der 2 ratio when samples are collected in bakeries. A specific interaction was found between Der 1 and Triticeae prolamins, the major seed storage proteins of wheat, barley, and rye<sup>3</sup> (insoluble in aqueous solu-

tions but soluble in ethanol-water mixtures). Most likely, this interaction underlies the specific depletion of Der 1 by cereal flours.

## METHODS

Dust samples and mite cultures were sieved through a 0.8 mm vibrating screen and then extracted in phosphate-buffered saline buffer, pH 7.4, supplemented with 0.2% wt/vol bovine serum albumin. The extracts were centrifuged, filtered through a 0.45  $\mu$ m pore-size filter, and then stored at  $-20^{\circ}$  C. Der 1 and Der 2 levels were determined by ELISA with monoclonal antibodies specific for Der p 1 and Der 2 group allergens. After appropriate dilutions of the extracts were incubated, specific rabbit polyclonal antisera were added, and then goat anti-rabbit IgG-purified antibodies conjugated with peroxidase. The enzymatic activity was then determined by using phenylenediamine as substrate.

Cysteine protease activity was analyzed with Resorfin-labeled casein (Boehringer Mannheim, Mannheim, Germany) essentially according to the manufacturer's instructions. Assays were carried out under conditions that prevented other proteolytic activities. For inhibition experiments, samples were preincubated for 1 hour at  $37^{\circ}$  C.

Wheat (*Triticum aestivum* cultivar Chinese Spring) flour was extracted with 0.5 mol/L NaCl (2 $\times$ ; 1:5 wt/vol; 1 hour;  $4^{\circ}$  C), and after washing the residue with distilled water, further extracted with 70% vol/vol ethanol (2 $\times$ ;

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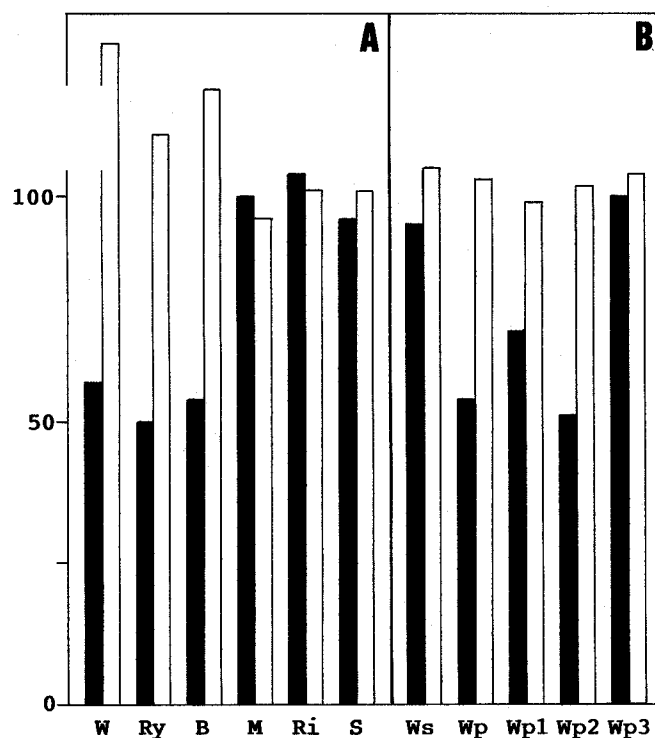


**FIG. 1. A,** Levels of Der p 1 (filled bars) and Der p 2 (open bars) in *D. pteronyssinus* extracts incubated with prolamin (alcohol-soluble proteins) preparations (1.0 mg/assay) from different cereals (W, Wheat; Ry, rye; B, barley; M, maize; Ri, rice) and soybean (S). Percent values with respect to control (only mite extracts) samples (2.8  $\mu$ g/ml of Der p 1 and 0.70  $\mu$ g/ml of Der p 2). **B,** Levels of Der p 1 (filled bars) and Der p 2 (open bars) in *D. pteronyssinus* extracts incubated with the following samples: (1.0  $\mu$ g/assay) from wheat flour: 0.5 mol/L NaCl extract (albumins + globulins; Ws); prolamin (ethanol extract; Wp), and gel-filtration fractions of the prolamin preparation (Wp1-Wp3; Wp3 corresponds to nonprolamin fractions with relative molecular mass <25,000). Percent values with respect to control samples (2.23  $\mu$ g/ml of Der p 1 and 0.51  $\mu$ g/ml of Der p 2).

1:5 wt/vol; 1 hour; room temperature). The prolamin preparation (ethanol extract) was then fractionated by gel filtration on Sephadex G-100 (Pharmacia, Uppsala, Sweden) under dissociating conditions. Ethanol extracts were also obtained from commercial barley, rye, maize, rice, and soybean samples. Purified Der p 1, either cold or labeled with iodine 125, was used when required.

## RESULTS

In routine screenings of dust samples with monoclonal antibodies specific for Der p 1 and Der 2, high titers of Der 2 (5.3 to 9.4  $\mu$ g/gm of dust), but only trace amounts of Der p 1 (<0.02  $\mu$ g/gm of dust), were detected in samples collected in bakeries. Because *D. pteronyssinus* was found to be the major mite in these samples (23% to 42% of 662 to 780 total mites per gram of dust), the possibility of a specific depletion of Der p 1 was investigated. Wheat flour and purified mites were extracted simultaneously, and the levels of Der p 1 and Der p 2, as well as the level of cysteine protease activity

(the enzymatic activity of Der p 1), were determined (Table I). Both the level of Der p 1 and the proteolytic activity appeared greatly decreased when mites and wheat flour were coextracted. Separate extraction of mites and flour, followed by mixing both extracts, resulted in a drastic decrease of proteolytic activity but not of Der p 1 (not shown).

To determine whether Der p 1 was selectively bound to insoluble flour components when mites and flour were coextracted,  $^{125}$ I-labeled Der p 1 was incubated with the mixture before extraction. After centrifugation, 70% of the label was recovered in the precipitate (whereas this value was only 3% when flour was not present). To investigate the specificity of this interaction, ethanol extracts (prolamins) from various cereals and one legume were prepared and mixed with mite extracts. Fig. 1, A shows the amount of Der p 1 and Der p 2 in these mixtures. Only flours from the Triticeae family

**TABLE 1.** Allergen levels and cysteine protease activity of extracts from wheat flour and mites and simultaneous extraction of purified mites and wheat flour

|                              | Allergen levels<br>( $\mu\text{g/ml}$ ) |                 | Enzymatic activity |                   |
|------------------------------|-----------------------------------------|-----------------|--------------------|-------------------|
|                              | Der p 1                                 | Der p 2         | A (574 nm)         | Relative activity |
| Mites 0.5 wt/vol             | 44.3 (SD = 3.8)                         | 16.4 (SD = 2.0) | 0.767 (SD = 0.025) | 100%              |
| Wheat flour 10% wt/vol       | <0.05                                   | 1.2 (SD = 0.2)  | 0.000              | 0%                |
| Wheat flour 10% + mites 0.5% | 5.2 (SD = 0.9)                          | 18.6 (SD = 0.9) | 0.146 (SD = 0.011) | 19%               |

Extraction was performed with phosphate-buffered saline buffer, 1% bovine serum albumin (mean values of triplicate experiments). SD, Standard deviation.

(wheat, barley, and rye) were able to bind specifically Der p 1 (1  $\mu\text{g/mg}$  of prolamin). However, Der p 1 levels were not affected by either the salt-soluble proteins or nonprolamin components of the wheat ethanol extract with relative molecular mass under 25,000<sup>4</sup> (Fig. 1, B).

## DISCUSSION

The clinical importance of an accurate mite allergen quantitation has been emphasized in the literature. We report here that a specific depletion of Der 1 allergen occurs when flour derivatives are in contact with dust. This fact should be considered when performing evaluations of allergenic exposures for different dust samples. Our data indicate that house dust mite infestation of flours has been underestimated when allergens of the Der 1 group were used as a monitoring parameter. Furthermore, the results presented here suggest that the level of Der 2 is a better reference parameter than that of Der 1, because no specific depletion has been found for the former allergen group and because of its greater physicochemical stability.

On the other hand, house dust mites are widely used in allergy diagnosis and treatment. Growth media have been optimized by each manufacturer and may include human and other mammalian derivatives, cereal flours, wheat germ, and other materials. When various house dust mite raw materials from different suppliers were evaluated, we found extreme variations in the Der 1/Der 2 ratio, which is in agreement with data reported by another group.<sup>5</sup> The depletion of Der 1 reported here could explain, at least in part, this variation.

Our results indicate that there is a specific

interaction between Der p 1 and wheat prolamins, which may explain the depletion of Der p 1 observed in dust samples from bakeries. To our knowledge, this is the first report of a specific interaction between an allergen and prolamins, the major seed storage proteins from wheat. The similarity of amino acid sequences and chemical properties among Triticeae prolamins may explain their similar behavior toward Der p 1. Prolamins from other cereals with different primary structures,<sup>3</sup> as well as ethanol-soluble proteins from soybean, do not exert any detectable effect on Der p 1.

The proteolytic activity of Der p 1 is inhibited by soluble components from wheat. However, it can be inferred from our data that these inhibitors are not relevant in sequestering Der p 1, as shown by the null effect of salt-soluble preparations (albumins and globulins), which include these inhibitors.

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## REFERENCES

1. Platts-Mills TAE, Chapman MD. Dust mites: immunology, allergic disease, and environmental control. *J ALLERGY CLIN IMMUNOL* 1987;80:755-75.
2. Bousquet J, Barber D, Campbell A, Martin S. Indoor allergen exposure. Analysis of mite allergen content from 539 European homes. *Allergy* 1992;12:61.
3. Shewry PR, Tatham AS. The prolamin storage proteins of cereal seeds: structure and evolution. *Biochem J* 1990;267:1-12.
4. Aragoncillo C, Sanchez-Monge R, Salcedo G. Two groups of low molecular weight hydrophobic proteins from barley endosperm. *J Exp Botany* 1981;32:1279-86.
5. Ford AW, Rawle FC, Lind P, Spielsman FTM, Løwenstein H, Platts-Mills TAE. Standardization of *D. pteronyssinus*. Assessment of potency and allergen content in ten coded extracts. *Int Arch Allergy Appl Immunol* 1985;76:58-67.